

Supplementary Data 1. Values for podosome and cell analysis

Fig. 2G	Mean velocity of podosome clusters [µm/min]			
Sample	Values	Mean ± SD		
control siRNA	24	0.3099 ± 0.1841		
LSP1 siRNA #1	24	0.8719 ± 0.5838		
LSP1 siRNA #2	24	0.7312 ± 0.4697		
One-way analysis of variance				
P value			0.0001	
P value summary			***	
Are means signif. different? (P < 0.05)			Yes	
Number of groups			3	
F			10.34	
R square			0.2307	
ANOVA Table		SS	df	MS
Treatment (between columns)		4.105	2	2.053
Residual (within columns)		13.69	69	0.1984
Total		17.8	71	
Bonferroni's Multiple Comparison Test		Mean Diff,	t	Summary
control vs LSP1 #1		-0.562	4.37	****
control vs LSP1 #2		-0.4213	3.276	**

Fig. 2H	Podosome lifetime [min]		
Sample	Values	Mean ± SD	
control siRNA	24	9.921 ± 2.843	
LSP1 siRNA #1	24	6.560 ± 2.465	
LSP1 siRNA #2	24	6.554 ± 1.441	
One-way analysis of variance			
P value			< 0.0001
P value summary			****
Are means signif. different? (P < 0.05)			Yes
Number of groups			3
F			16.72
R square			0.3264
ANOVA Table		SS	df
Treatment (between columns)		181	2
Residual (within columns)		373.5	69
Total		554.5	71
Bonferroni's Multiple Comparison Test		Mean Diff,	t
control vs LSP1 #1		3.360	5.003
control vs LSP1 #2		3.367	5.013

Fig. 2M	Mean velocity of cells [µm/min]			
Sample	Values	Mean ± SD		
control siRNA	24	0.2380 ± 0.0977		
LSP1 siRNA #1	24	0.3329 ± 0.2569		
LSP1 siRNA #2	24	0.3521 ± 0.2486		
One-way analysis of variance				
P value			0.1488	
P value summary			ns	
Are means signif. different? (P < 0.05)			No	
Number of groups			3	
F			1.958	
R square			0.05372	
ANOVA Table		SS	df	MS
Treatment (between columns)		0.00004982	2	0.00002491
Residual (within columns)		0.0008776	69	0.00001272
Total		0.0009274	71	
Bonferroni's Multiple Comparison Test		Mean Diff,	t	Summary
control vs LSP1 #1		-0.001582	1.537	ns
control vs LSP1 #2		-0.001903	1.848	ns

Fig. 2N	Mean cell area [μm²]			
Sample	Values	Mean ± SD		
control siRNA	24	1456 ± 407		
LSP1 siRNA #1	24	2226 ± 1025		
LSP1 siRNA #2	24	2771 ± 1506		
One-way analysis of variance				
P value			0.0003	
P value summary			***	
Are means signif. different? (P < 0.05)			Yes	
Number of groups			3	
F			9.020	
R square			0.2073	
ANOVA Table		SS	df	MS
Treatment (between columns)		20950000	2	10480000
Residual (within columns)		80140000	69	1161000
Total		101100000	71	
Bonferroni's Multiple Comparison Test		Mean Diff,	t	Summary
control vs LSP1 #1		-770.4	2.476	*
control vs LSP1 #2		-1315	4.227	***

Fig. 2O	Podosome distribution (clusters) [%]			
Sample	Values	Mean ± SD		
control siRNA	3	19.07 ± 7.067		
LSP1 siRNA #1	3	40.17 ± 6.250		
LSP1 siRNA #2	3	48.17 ± 5.244		
One-way analysis of variance				
P value			0.0032	
P value summary			**	
Are means signif. different? (P < 0.05)			Yes	
Number of groups			3	
F			17.46	
R square			0.8534	
ANOVA Table		SS	df	MS
Treatment (between columns)		1356	2	678.0
Residual (within columns)		233.0	6	38.84
Total		1589	8	
Bonferroni's Multiple Comparison Test		Mean Diff,	t	Summary
control vs LSP1 #1		-21.10	4.147	*
control vs LSP1 #2		-29.10	5.719	**

Fig. 2W	Cell shape analysis (AR ≥ 1.3 , circ. ≤ 0.8) [%]			
Sample	Values	Mean ± SD		
control siRNA	3	19.21 ± 7.285		
LSP1 siRNA #1	3	59.73 ± 9.941		
LSP1 siRNA #2	3	53.87 ± 8.454		
One-way analysis of variance				
P value			0.0084	
P value summary			**	
Are means signif. different? (P < 0.05)			Yes	
Number of groups			3	
F			11.78	
R square			0.7971	
ANOVA Table		SS	df	MS
Treatment (between columns)		2877	2	1439
Residual (within columns)		732.6	6	122.1
Total		3610	8	
Bonferroni's Multiple Comparison Test		Mean Diff,	t	Summary
control vs LSP1 #1		-40.52	4.491	**
control vs LSP1 #2		-34.66	3.842	*

Fig. 3E, 3L	Podosome oscillations [number of peaks]			
Sample	Values	Mean $\pm$ SD	Unpaired t-test	
			t	df
control GFP	30	2.900 $\pm$ 0.8030	6.143	58
LSP1-GFP	30	4.333 $\pm$ 0.9942		
control siRNA	30	3.133 $\pm$ 0.7761	2.128	58
LSP1 siRNA #2	30	2.600 $\pm$ 1.133		

Fig. 3F, 3M	Podosome oscillations [height of peaks]			
Sample	Values	Mean $\pm$ SD	Unpaired t-test	
			t	df
control GFP	87	20.46 $\pm$ 9.525	2.046	215
LSP1-GFP	130	17.36 $\pm$ 11.77		
control siRNA	93	21.42 $\pm$ 12.22	4.473	169
LSP1 siRNA #2	78	32.78 $\pm$ 20.55		

Fig. 3P	Podosome protrusion force [nN]			
Sample	Values	Mean $\pm$ SD	Unpaired t-test	
			t	df
control siRNA	21	2.451 $\pm$ 0.7480	2.742	34
LSP1 siRNA #2	15	1.704 $\pm$ 0.8811		

Fig. 4E	Myosin IIA fluorescence intensity at podosomes			
Sample	Values	Mean ± SD		
control siRNA	15	54.14 ± 21.31		
LSP1 siRNA #1	15	31.81 ± 16.25		
LSP1 siRNA #2	15	35.91 ± 11.93		
One-way analysis of variance				
P value			0.0018	
P value summary			**	
Are means signif. different? (P < 0.05)			Yes	
Number of groups			3	
F			7.394	
R square			0.2604	
ANOVA Table		SS	df	MS
Treatment (between columns)		4241	2	2121
Residual (within columns)		12045	42	286.8
Total		16286	44	
Bonferroni's Multiple Comparison Test		Mean Diff,	t	Summary
control vs LSP1 #1		22.34	3.612	**
control vs LSP1 #2		18.23	2.948	*

Fig. 4F	F-actin fluorescence intensity at podosomes			
Sample	Values	Mean ± SD		
control siRNA	15	68.34 ± 22.16		
LSP1 siRNA #1	15	65.01 ± 33.07		
LSP1 siRNA #2	15	68.10 ± 37.43		
One-way analysis of variance				
P value			0.9492	
P value summary			ns	
Are means signif. different? (P < 0.05)			No	
Number of groups			3	
F			0.05224	
R square			0.002481	
ANOVA Table		SS	df	MS
Treatment (between columns)		104.0	2	51.99
Residual (within columns)		41799	42	995.2
Total		41903	44	
Bonferroni's Multiple Comparison Test		Mean Diff,	t	Summary
control vs LSP1 #1		3.339	0.2898	ns
control vs LSP1 #2		0.2420	0.02101	ns

Fig. 4G	Myosin IIA ROI area [μm^2]			
Sample	Values	Mean $\pm$ SD		
control siRNA	15	1.669 $\pm$ 0.5050		
LSP1 siRNA #1	15	1.824 $\pm$ 0.7771		
LSP1 siRNA #2	15	1.968 $\pm$ 0.5040		
One-way analysis of variance				
P value			0.4118	
P value summary			ns	
Are means signif. different? (P < 0.05)			No	
Number of groups			3	
F			0.9063	
R square			0.04137	
ANOVA Table		SS	df	MS
Treatment (between columns)		0.6723	2	0.3362
Residual (within columns)		15.58	42	0.3709
Total		16.25	44	
Bonferroni's Multiple Comparison Test		Mean Diff,	t	Summary
control vs LSP1 #1		-0.1557	0.7001	ns
control vs LSP1 #2		-0.2993	1.346	ns

Fig. 4H	F-actin ROI area [μm^2]			
Sample	Values	Mean $\pm$ SD		
control siRNA	15	0.4534 $\pm$ 0.2088		
LSP1 siRNA #1	15	0.4103 $\pm$ 0.1533		
LSP1 siRNA #2	15	0.5610 $\pm$ 0.1795		
One-way analysis of variance				
P value			0.0766	
P value summary			ns	
Are means signif. different? (P < 0.05)			No	
Number of groups			3	
F			2.733	
R square			0.1151	
ANOVA Table		SS	df	MS
Treatment (between columns)		0.1810	2	0.09048
Residual (within columns)		1.391	42	0.03311
Total		1.572	44	
Bonferroni's Multiple Comparison Test		Mean Diff,	t	Summary
control vs LSP1 #1		-0.04311	0.6488	ns
control vs LSP1 #2		-0.1077	1.621	ns

Fig. 4L	PLA spots / cell			
Sample	Values	Mean $\pm$ SD	Unpaired t-test	
			t	df
IgG + myo IIA	130	2.43 $\pm$ 4.76	9.04	258
LSP1 + myo IIA	130	72.98 $\pm$ 88.84		

Fig. 7E	Correlation and linear regression of actin isoforms	
Sample	Pearson r	R ²
α -cardiac actin	0.3934	0.1548
β -actin	- 0.8055	0.6488
γ -actin	- 0.6515	0.4244

Fig. 8B	F-actin co-sedimentation [P / (S+P)] [%]			
Sample	Values	Mean $\pm$ SD	Unpaired t-test	
			t	df
LSP1 + β -actin	5	84.00 $\pm$ 6.015	11.77	8
LSP1 + α -cardiac actin	5	44.58 $\pm$ 4.465		

Fig. 9M	Mean cell area [μm^2]			
Sample	Values	Mean $\pm$ SD		
control siRNA	120	1552 $\pm$ 747.1		
LSP1 siRNA #1	120	1548 $\pm$ 728.0		
LSP1 siRNA #2	120	1794 $\pm$ 1000		
One-way analysis of variance				
P value			0.0341	
P value summary			*	
Are means signif. different? (P < 0.05)			Yes	
Number of groups			3	
F			3.411	
R square			0.01880	
ANOVA Table		SS	df	MS
Treatment (between columns)		4743000	2	2372000
Residual (within columns)		247600000	356	695377
Total		252300000	358	
Bonferroni's Multiple Comparison Test		Mean Diff,	t	Summary
control vs LSP1 #1		4.116	0.03823	ns
control vs LSP1 #2		-242.1	2.244	ns

Fig. 9N	Podosome distribution (polarised) [%]			
Sample	Values	Mean ± SD		
control siRNA	3	45.70 ± 3.940		
LSP1 siRNA #1	3	27.30 ± 7.467		
LSP1 siRNA #2	3	24.00 ± 6.065		
One-way analysis of variance				
P value			0.0091	
P value summary			**	
Are means signif. different? (P < 0.05)			Yes	
Number of groups			3	
F			11.39	
R square			0.7915	
ANOVA Table		SS	df	MS
Treatment (between columns)		820.3	2	410.2
Residual (within columns)		216.1	6	36.02
Total		1036	8	
Bonferroni's Multiple Comparison Test		Mean Diff,	t	Summary
control vs LSP1 #1		18.40	3.755	*
control vs LSP1 #2		21.70	4.428	**

Fig. 9U	Cell shape analysis (AR ≥ 1.3 , circ. ≤ 0.8) [%]			
Sample	Values	Mean ± SD		
control siRNA	3	27.74 ± 9.415		
LSP1 siRNA #1	3	9.963 ± 6.314		
LSP1 siRNA #2	3	16.98 ± 5.114		
One-way analysis of variance				
P value			0.06	
P value summary			ns	
Are means signif. different? (P < 0.05)			No	
Number of groups			3	
F			4.662	
R square			0.6085	
ANOVA Table		SS	df	MS
Treatment (between columns)		480.8	2	240.4
Residual (within columns)		309.3	6	51.56
Total		790.1	8	
Bonferroni's Multiple Comparison Test		Mean Diff,	t	Summary
control vs LSP1 #1		17.77	3.031	*
control vs LSP1 #2		10.75	1.834	ns

Suppl. Fig. 4A	Western blot quantification of LSP1 knockdown [% vs control]				
Sample	Values	Mean $\pm$ SD	One sample t-test (theor.mean = 100)		
			t	df	P value
LSP1 #1	8	51.89 $\pm$ 15.78	8.622	7	< 0.0001
LSP1 #2	8	44.17 $\pm$ 16.30	9.687	7	< 0.0001

Suppl. Fig. 5G	Mean number of cells invading into 3D collagen I [% vs control]				
Sample	Values	Mean $\pm$ SD	One sample t-test (theor.mean = 100)		
			t	df	P value
LSP1 siRNA #1	3	166.00 $\pm$ 12.73	9.030	2	0.0120
LSP1 siRNA #2	3	150.60 $\pm$ 3.69	23.75	2	0.0018

Suppl. Fig. 5H	Mean value of invaded area [% vs control]				
Sample	Values	Mean $\pm$ SD	One sample t-test (theor.mean = 100)		
			t	df	P value
LSP1 siRNA #1	3	100.70 $\pm$ 5.508	0.2097	2	0.8534
LSP1 siRNA #2	3	97.00 $\pm$ 9.644	0.5388	2	0.6440

Suppl. Fig. 6G	PLA spots / cell			
Sample	Values	Mean $\pm$ SD	Unpaired t-test	
			t	df
IgG + pan-actin	140	1.12 $\pm$ 3.37		
LSP1 + pan-actin	140	175.90 $\pm$ 148.50	13.92	278

Suppl. Fig. 9B	Western blot quantification of α -cardiac actin knockdown [% vs control]				
Sample	Values	Mean $\pm$ SD	One sample t-test (theor.mean = 100)		
			t	df	P value
α -card act. /GAPDH	4	66.44 $\pm$ 12.16	5.522	3	0.0117
β -act. /GAPDH	4	87.68 $\pm$ 7.881	3.128	3	0.0522
γ -act. /GAPDH	4	103.0 $\pm$ 18.84	0.3159	3	0.7728
LSP1 /GAPDH	4	99.84 $\pm$ 16.15	0.02010	3	0.9852

Suppl. Fig. 9B	Western blot quantification of β -actin knockdown [% vs control]				
Sample	Values	Mean $\pm$ SD	One sample t-test (theor.mean = 100)		
			t	df	P value
α -card act. /GAPDH	4	75.19 $\pm$ 21.15	2.346	3	0.1006
β -act. /GAPDH	4	39.58 $\pm$ 18.36	6.580	3	0.0071
γ -act. /GAPDH	4	120.3 $\pm$ 31.99	1.270	3	0.2937
LSP1 /GAPDH	4	89.14 $\pm$ 21.67	1.002	3	0.3901